EXHIBIT A

Comments re: Protest of Reissue of US 5,750,338
Dr. Kary B. Mullis, Jan 23, 2001 DRAFT

The invention described in '338 was conceived as a method of bringing some level of specificity to nucleic acid amplification schemes such as those employing E. coli RNA polymerase lacking the sigma subunit, or the same enzyme following random hexamer primed DNA synthesis, or a combination of enzymes including Q-Beta-replicase, as described in the examples, where the amplification step was itself non-specific. Nonetheless, some variant of this method could have obviously been employed prior to a specific nucleic acid amplification such as the polymerase chain reaction.

In fact it was probably at least once, but finding the reference might not be easy; it wouldn't have been a landmark development; it might have been done several times even and people doing it would not have thought to claim it as a new development, just business as usual. The concept would not have been considered novel in 1987.

**BUT LOOK AT THIS!!!!!!**

**People were and still are snatching mRNAs out of extracts with oligo-dT-cellulose every day, eluting them, and then doing RT-PCR on them. That's the same sort of thing. I think this fairly common process reads directly on Claim 1, A, B, and C!!. Also claims 2-5, 7-11, and all their derivatives, leaving claim 6 and maybe 12, which DNA restricted process somebody must have published somewhere as mentioned above]]**

On another tack,
The use of nested primers in pcr is in essence a pre-purification of the sample in the first stage, and the specificity of this pre-purification is dependent on specific sequence hybridization as is the specificity of any pcr reaction. The mechanism for purification is not dependent on removal of non-intended sequences by use of a solid support, but by the principle of overwhelming amplification of the target sequence, in other words, the impurities are eliminated by default—by not being amplified. The effect is the same and the purpose for the first stage amplification is to improve the specificity of the second stage amplification by enriching the DNA sample for the desired target. The need for such a first stage amplification obviously arose from the fact that pcr reactions were not perfect discriminators before or after 1987—nothing in the universe is, it could easily go without saying—and certainly nobody very familiar with pcr in 1987 was under the erroneous impression that in certain cases, the reaction was not absolutely specific. A number of post-amplification hybridization techniques, as well as non-hybridization techniques such as running gels, for dealing with non-specificity and signal generation were employed and still are.

Mullis 1/6/01
EXHIBIT
213

**I think I would be infringing claim 20 if I were to provide a kit to:**

- **(a)** Purify DNA from a tube of blood by separating out the buffy coat. homogenize the cells, isolate DNA from the extract using any of a dozen well known COMMERCIAL methods, available as kits.
- **(b)** Amplify with PCR, also available as a kit.
- **(c)** Put the amplified target on a membrane (lots of kits for this) and
- **(d)** Probe with a labeled oligo. (also kits here).

**The subordinant kit claims are more specific, but I think anyone can see that this patent is transparently non-inventive and designed not for the purpose of teaching anybody how to do anything, but simply to lay claim to and profit from what is already being done, and was obvious to anyone even marginally skilled in the art for over a decade.**